Apicomplexa

William C Marquardt, *Colorado State University, Fort Collins, Colorado, USA*

CA Speer, *Montana State University, Bozeman, Montana, USA*

The phylum Apicomplexa includes several thousand parasitic protists that cause major diseases of mankind and of domestic and companion animals. Among the diseases that they cause are malaria, coccidiosis, toxoplasmosis, sarcocystosis, cyclosporosis, cryptosporidiosis and piroplasmosis.

Secondary article

Article Contents

- Introduction
- Characterization
- Place in Overall Taxonomic Scheme
- Phylogenetic and Evolutionary Considerations
- Host–Parasite Interactions
- Major Taxa and Important Species

Introduction

The Apicomplexa comprises a phylum of protists that have structures referred to as the apical complex and in which the life cycles have both asexual and sexual phases. All members are parasitic. Included in the phylum are several thousand described species that parasitize members of all of the major animal phyla. They are parasites of common invertebrate phyla such as molluscs, arthropods and annelids as well as of some lesser-known phyla. Apicomplexans are parasites of members of all the classes of the phylum Chordata including sea squirts or tunicates, fish, amphibia, reptiles, birds and mammals.

Members of the Apicomplexa cause some of the most devastating diseases of mankind and cause enormous economic losses in domesticated animals and poultry. The most prominent apicomplexans are *Plasmodium* spp., which cause malaria in humans; even after one hundred years of concerted control programmes, malaria still takes more than 2 million human lives each year. Coccidiosis is a complex of intestinal diseases of livestock and poultry that are common wherever animals are raised for food and wool. Global losses to coccidiosis reach into many millions of dollars (US) annually. Poultry cannot be raised in current high concentration methods without the continuous use of drugs to prevent the development of the causative agents of coccidiosis. Piroplasmoses are tick-borne diseases that affect livestock on all continents and may be the single most important group of infectious agents of grazing animals.

Characterization

Members of the phylum have one or more elements of the apical complex (**Figure 1**) at some stage of the life cycle: polar ring, rhoptries, micronemes and conoid. Sexuality is by syngamy. Multiplication is by schizogony or endodyogeny; $9 + 2$ flagellar form is present in some gametes; locomotion is by body flexion or gliding; subpellicular tubules are present in most species. Feeding is by osmotrophy or phagotrophy, the latter by a cytostome (**Figure 2**), often referred to as a micropore. All species are parasitic.

Schizogony is an asexual process of multiplication in which multiple nuclear replications take place without

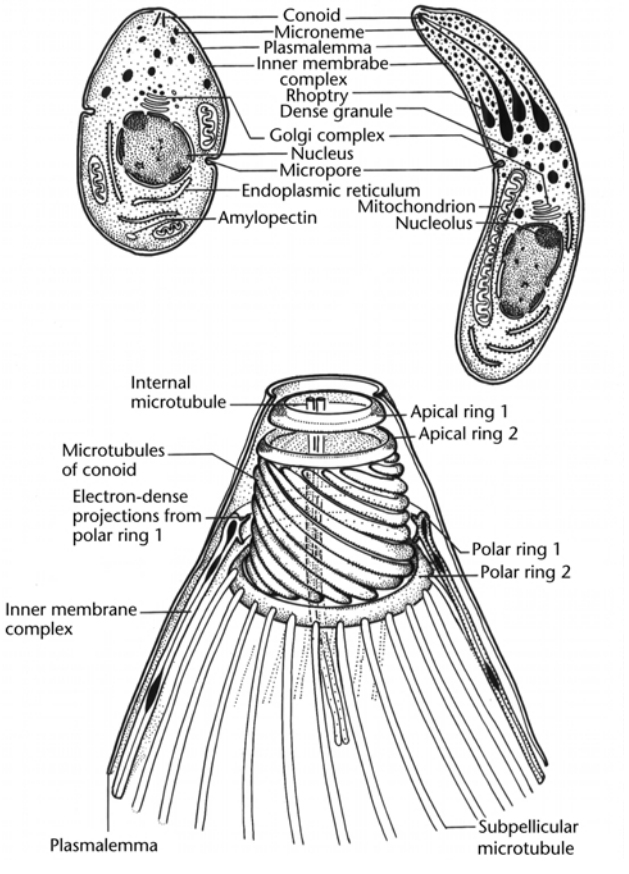
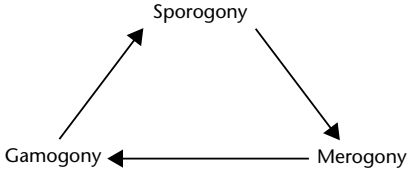


Figure 1 Structure of apicomplexans as revealed by electron microscopy. Upper left: a metrocyte, or mother cell, in which the elements of the apical complex are minimally expressed. Upper right: a zoite in which all of the elements of the apical complex are seen. Lower: A detailed view of the conoid and associated elements such as the polar rings and the subpellicular tubules which extend about two-thirds of the length of the zoite. (Courtesy of C. A. Speer.)

cytokinesis until all the progeny have formed. Then, cytokinesis occurs simultaneously forming all of the progeny (**Figure 3**). Endodyogeny is a process in which two progeny form within a parental cell (**Figure 4**); when the offspring are fully formed, the parental cell breaks down, releasing them.

Apicomplexans have three stages in their life cycles:



Note that the cycle runs in only one direction. The organism is genetically programmed for one stage to follow the other.

Eimeria tenella of the chicken is a typical apicomplexan that has its stages in the intestinal tract of its host (**Figure 5**). Sporogony takes place outside of the host when oocysts are shed with the faeces. When an infective oocyst (**Figure 6**) is eaten, merogony takes place in the cells of the large intestine. After three merogonies, gamogony takes place with the formation of macro- and microgamonts. Upon syngamy, the cycle is completed with the formation of oocysts.

Sporogony is a schizogonic process in which many sporozoites are the product and which usually takes place within an oocyst (**Figure 6**). The sporozoite is the infective stage in homoxenous parasites and in heteroxenous organisms such as *Plasmodium* spp. Merogony is also a schizogonic process and the main one by which the apicomplexans multiply within a host. In malaria, this process occurs first in the liver and then in the red blood cells. Gamogony results in the formation of gametocytes, which undergo syngamy to form a new individual. In most instances, there are distinctly male and female gametes, but in many of the gregarines, isogametes are formed.

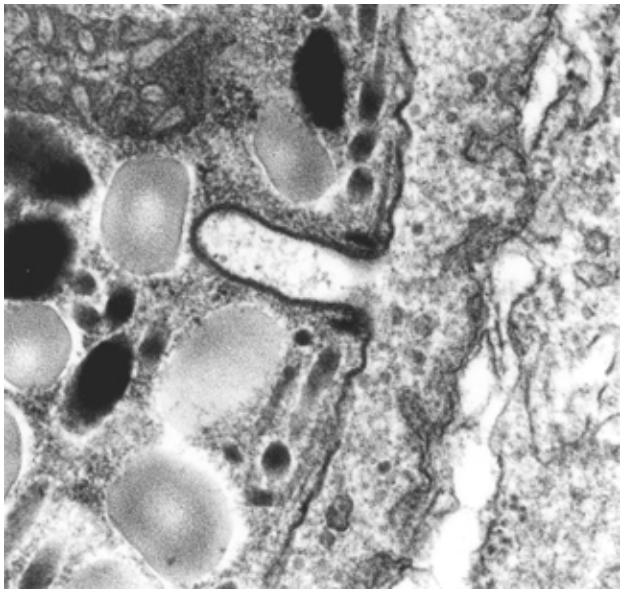


Figure 2 The cytostome or micropore of *Toxoplasma gondii* as seen in transmission electron microscopy. The cytostome ingests food provided by the parasite's host cell. (Courtesy of C. A. Speer.)

Place in Overall Taxonomic Scheme

Protistan (protozoan and protophytan) classification has undergone and continues to undergo radical changes. Despite what has occurred in other taxa, the phylum Apicomplexa remains a discrete group. The organelles that comprise the apical complex as well as the life cycles and mode of division are quite consistent within the group. The exception is the class Perkinsea whose inclusion within the phylum remains problematic. Only three species have been described and they have been included in the phylum Apicomplexa based on a structure that appears to be a partial conoid but molecular studies will be needed to clarify the status of this enigmatic group.

Phylogenetic and Evolutionary Considerations

In classification and in evolutionary studies, one tries to determine which group serves as an ancestor for another. In the case of protists generally, reaching such conclusions was rather difficult until the development of electron microscopy and molecular methods. The evidence now suggests that Apicomplexa arose from a mostly marine taxon commonly referred to as the dinoflagellates. Most of its members are free-living, but some are symbiotic in metazoa. The conclusion that the apicomplexans are derived from the dinoflagellates is based on (1) the types of hosts infected, (2) the mode of division (schizogony) in some members, (3) cyst formation, and (4) the nucleic acid sequence of the small subunit rRNA gene. A peculiar multiple-membrane bound, DNA-containing, plastid-like organelle (originally called a Golgi adjunct) has been described in several apicomplexans. Phylogenetic analysis of the plastid gene (called *tufA*) of *Toxoplasma gondii* has placed its organellar genome with cyanobacteria and plastids, especially green algal plastids. An intimate association between the plastid and the nuclear spindle has also been found in some apicomplexans, indicating that the plastid may play a role in nuclear division (Speer and Dubey, 1999).

Within the phylum Apicomplexa itself, the following is generally agreed upon as the evolutionary sequence for the

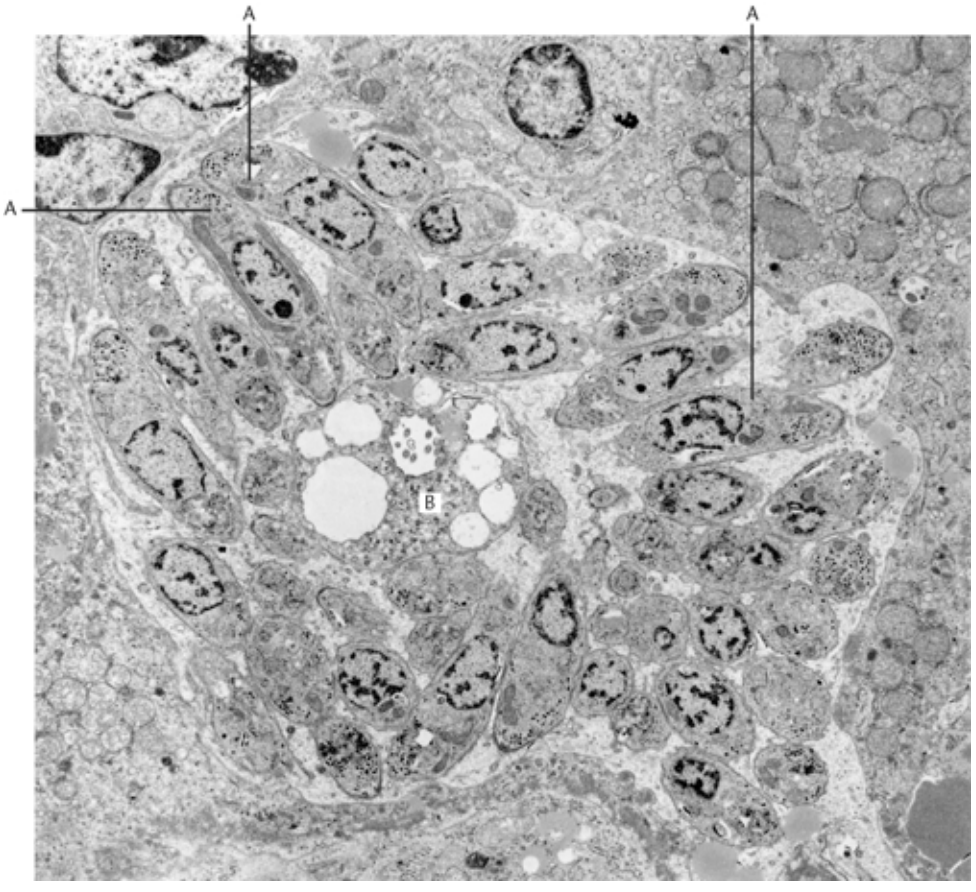


Figure 3 A meront of *Sarcocystis* sp. in a hepatocyte of a mouse as seen in transmission electron microscopy. (A) Merozoites that have completed budding from (B) a centrally located residual body. (Courtesy of C. A. Speer.)

classes (excluding Perkinsea). Probably first were gregarines, or something like them. These organisms infect invertebrates and most are coelozoic although some groups are cytozoic. The long evolutionary history of the apicomplexans can be inferred from the fact that members of the genus *Eimeria* parasitize tunicates or sea squirts, marine chordates from which fishes began to diverge perhaps 500 million years ago. *Eimeria* spp. infect the intestines of all classes of vertebrates: fish, amphibia, reptiles, birds and mammals. *Eimeria* spp., with few exceptions show a high degree of host specificity, usually infecting only a few closely related species of hosts and suggesting a long coevolution.

The shift to a two-host life cycle, such as occurs facultatively in *Toxoplasma gondii*, probably occurred when a paratenic host was inserted into the life cycle. Two hosts eventually became obligatory, as in *Sarcocystis* and *Neospora*. Transmission by haematophagous arthropods (or other invertebrates) is the final step in the evolutionary sequence. In the Adeleina, the arthropod, often a mosquito, is eaten in order to infect the vertebrate. In Haemosporea and Piroplasmea transmission haematophagous arthropods inject the organisms.

Host–Parasite Interactions

Nearly all apicomplexans are cytozoic intracellular parasites. The gregarines usually have a holdfast or epimerite by which they attach to cells; as the organisms grow, the trophozoites become large enough to see with the naked eye and extend into the cavity of the organ. In *Plasmodium* spp., which cause malaria in humans, the stages in the mosquito are extracellular. The intracellular mode of life is often said to protect the organism from the immune response of the host, but the long association of apicomplexans and coevolution with their hosts also suggests that they parasitize the metabolic reactions of their host cells. The striking enlargement of the nuclei and nucleoli in host cells seen in certain coccidial infections

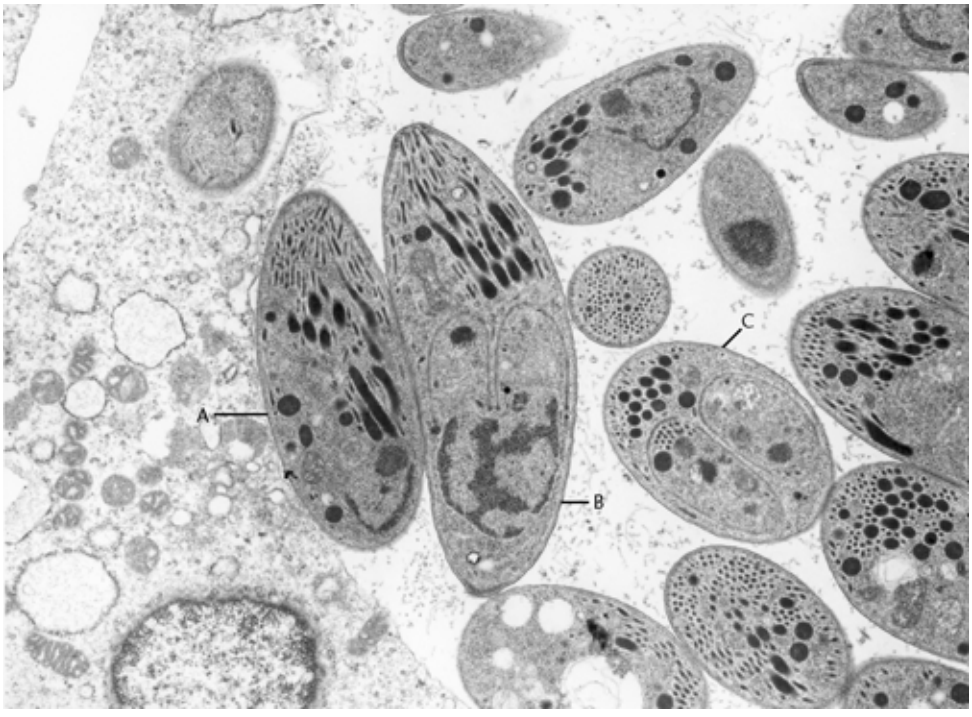


Figure 4 Endodyogeny in zoites of *Neospora caninum*, an economically important parasite of domestic animals. (A) A zoite not undergoing endodyogeny. (B) Partially formed progeny cells in which the nucleus is being divided into two progeny cells. (C) Two progeny cells completely formed and probably ready to be released. (Courtesy of C. A. Speer.)

suggests that the transcriptional activity of the host cells is increased, perhaps selectively.

In vitro cultivation

Cultivation of an infectious agent outside of its host allows the organism to be characterized and its mode of development can readily be studied. Advances in cultivating bacteria in the nineteenth century brought about enormous advances in knowledge of infectious diseases and in their control. The earliest attempts to culture apicomplexan parasites were made early in the twentieth century and involved *Plasmodium* spp., *Sarcocystis tenella*, *Gastrocystis gilruthi*, *Toxoplasma gondii* and *Eimeria* spp. These attempts were unsuccessful, but somewhat later, techniques for isolating and cultivating cells and tissues from vertebrates were refined. By the late 1940s, major discoveries led to the development of reliable methods of culturing vertebrate cells *in vitro*. Subsequently, techniques were developed that led to standardized procedures for the cultivation of many types of cells, to the development of highly defined culture media, and to pathogen-free culture media. Today, various culture media are available commercially, as are some apicomplexan parasites.

Techniques have now been developed for the *in vitro* cultivation of many apicomplexans including species of *Babesia*, *Plasmodium*, *Cryptosporidium*, *Eimeria*, *Theileria*, *Sarcocystis*, *Toxoplasma*, *Isospora*, *Hammondia* and others. *In vitro* cultivation of apicomplexans has led to information on antigens, parasite development, biochemistry, host–parasite interactions, host cell invasion and molecular biology.

A major advance concerning the cultivation of apicomplexans was made in 1976 by Trager and Jensen who succeeded in continuous cultivation of meronts of *Plasmodium falciparum*, the organism that causes malignant tertian malaria in humans. This discovery facilitated research in malaria immunology and biochemistry and is recognized as initiating the era of molecular biological research on malaria.

In a few instances, the complete life cycles of apicomplexans have been obtained *in vitro*. In most cases, when cultured cells are inoculated with sporozoites, apicomplexans develop only through the first-generation meronts or they multiply exclusively by endodyogeny. Some apicomplexans are facultative or obligately heteroxenous parasites (i.e. they require two hosts to complete their life cycles). Even in those that are monoxenous, the endogenous stages often develop in different sites and in different types of cells within the host. For example, first-generation meronts of *Eimeria bovis* develop in endothelial cells of the central lacteals of the ileal portion of the intestine, whereas second-generation meronts and gametocytes develop in glandular epithelial cells in the large intestine and caecum.

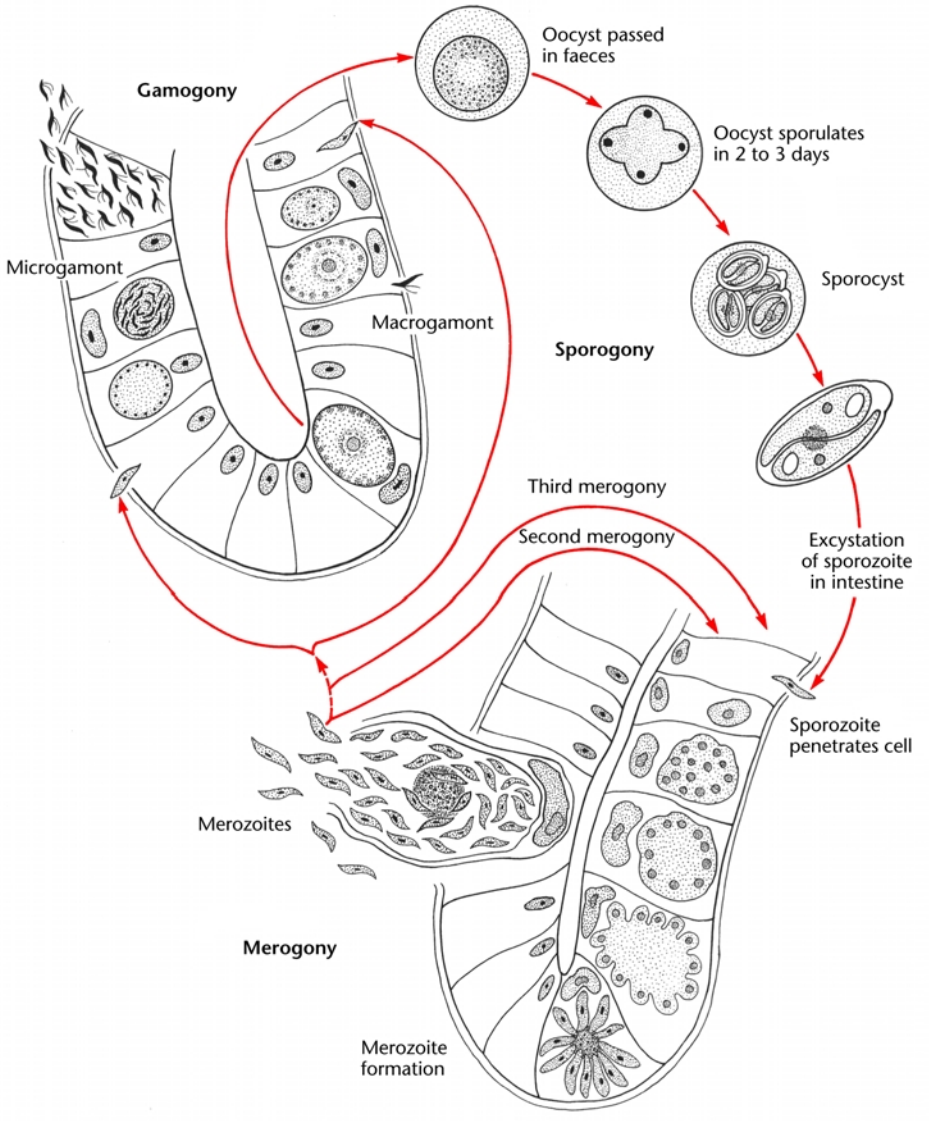


Figure 5 The life cycle of *Eimeria tenella* of the chicken. This apicomplexan is typical of those coccidia that parasitize the intestinal tracts of their hosts. Sporogony takes place outside of the host, and both merogony and gamogony occur within cells of the intestine. (From Marquardt *et al.*, 1999.)

Cultures inoculated with sporozoites of *E. bovis* develop only to first-generation meronts, but merozoites obtained from the intestinal tract of infected calves develop to gametocytes and oocysts in cultured cells. Further parasite development probably involves certain environmental conditions that are required for the stimulation of developmentally regulated genes, enabling the parasite to proceed to the next developmental phase.

The complete endogenous cycles of *Cryptosporidium parvum*, *Isospora suis* and certain *Eimeria* spp. from chickens have been obtained *in vitro*. Endodyogeny is the only form of asexual reproduction that occurs with *Toxoplasma gondii*, *Besnoitia* spp., *Hammondia* spp. and *Neospora caninum in vitro*. Meronts and gametocytes of these species have not been cultured *in vitro*, indicating that in these systems the appropriate cell types, cultural conditions or components of the medium are lacking.

Research has shown that lowering the pH of the culture medium or adding parasite-specific antibodies to the culture medium induces tachyzoites of *T. gondii*, *Hammondia* spp. and *N. caninum* to form cysts containing bradyzoites. Techniques have also been developed for the isolation and cultivation of apicomplexans from tissues of infected hosts. For example, apicomplexans such as *T. gondii*, *N. hughesi* and *Sarcocystis neurona* (the latter

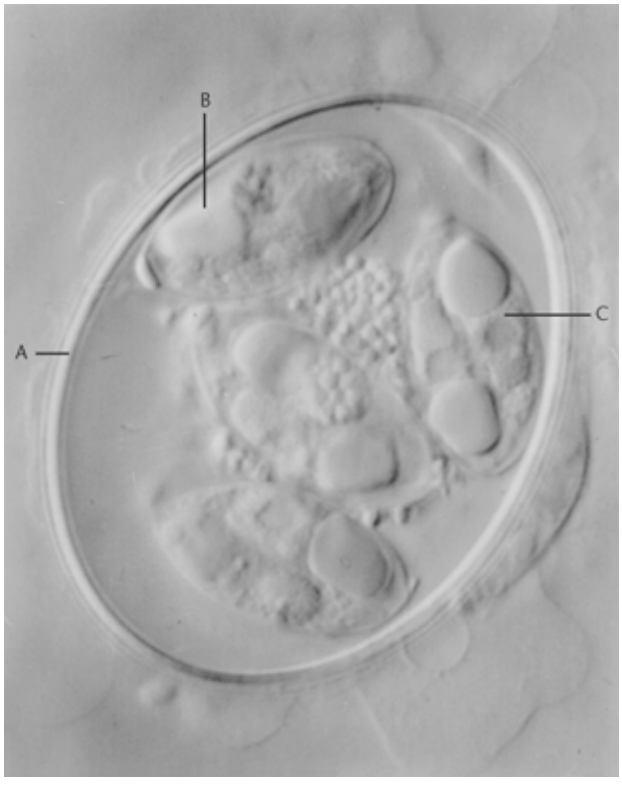


Figure 6 An oocyst of *Eimeria magna* of the rabbit, *Oryctolagus cuniculi*, as seen in interference contrast light microscopy. The oocyst is infective if ingested by a rabbit. In the genus *Eimeria*, each oocyst has four sporocysts each of which contains two sporozoites. (A) Oocyst wall. (B) Sporocyst. (C) Sporozoite. (Courtesy of C. A. Speer.)

causes equine protistan myeloencephalitis) can be isolated from brain and spinal cord tissue and grown *in vitro*.

Penetration of host cells

Zoites of many apicomplexans are highly motile, undergoing flexing, gliding, and pivoting, and probing with their anterior ends. Direct microscopic observation has revealed that host cell penetration is usually preceded by gliding movements and probing of the host cell surface with the parasite's anterior end. Parasite motility and cell penetration are powered by an actin–myosin based motor in the parasite that is characterized by the rearward capping of surface membrane proteins that propels the parasite forward in a helical path.

In general, the events involved in zoite attachment and penetration of host cells include: (1) gliding of the zoite immediately before attachment and penetration; (2) probing and attachment to the host cell with the conoid; (3) indentation of the host cell plasmalemma; (4) formation of a junction that moves posteriorly as the zoite penetrates the host cell; and (5) partial exocytosis of micronemes, rhoptries, and dense granules. *In vitro*, zoites can penetrate a variety of cell types from a wide range of hosts, indicating that the biochemical receptors involved in attachment and penetration involve either the presence of a highly conserved host cell receptor or a variety of different receptors recognized by the parasite. In *T. gondii*, tachyzoite attachment to host cells involves ubiquitous receptors consisting of a parasite laminin–host cell laminin receptor, lectins, and a glycosylated receptor, SAG1. Attachment of tachyzoites to host cells can be inhibited by antibodies and is saturable in peptide competition assays. Tachyzoites made deficient in SAG1 bind significantly less to host cells than do wild-type tachyzoites. In addition, micronemes of *T. gondii* tachyzoites contain at least three specific proteins (MIC1–3), some of which are exocytosed during host cell penetration and may be involved in host cell receptor recognition and attachment.

Proteins containing one or more copies of the type I repeat of human platelet thrombospondin (TSP1) are necessary for parasite motility and invasion of host cells. Members of the vertebrate TSP family are adhesive molecules involved in cell–cell and cell–matrix interactions and blood coagulation. The presence of TSP1 repeats in apicomplexans appears to enable these parasites to mimic host cell molecules, such as TSP1, F-spondin, properdin and C6-C9 of the complement cascade, and use their natural ligands during movement over the surface of host cells and during host cell penetration. Among the Apicomplexa, proteins related to motility include all of the proteins released by sporozoites of *Plasmodium* species, the thrombospondin-related adhesive protein (TRAP) of *P. falciparum* and other *Plasmodium* species, the micronemal proteins of *Eimeria maxima* (Em100) and *E. tenella* (Etp100), micronemal protein 2 (MIC2) of *Toxoplasma gondii* and the TRAP of *Cryptosporidium parvum*. Based on studies with these apicomplexans, a molecular model has been proposed for TRAP function in zoite motility. TRAP is stored within micronemes at the sporozoite apical end and is continuously translocated to the zoite plasmalemma. TRAP binds to extracellular host cell ligands and acts as a link with the parasite molecular motor. A network of actin–myosin lies in direct contact with the outer face of the zoite plasmalemma. Backward sliding of the myosin–TRAP–ligand complexes along longitudinal actin filaments pushes the zoite body forward. This causes accumulation at the posterior of the zoite of TRAP complexes, which dissociate leaving a trail of TRAP.

Apicomplexans have also been found to enter cells by being phagocytosed or by purely mechanical mechanisms. For example, sporozoites of *E. bovis* that were completely surrounded by host cell membranes and cytoplasm have been observed by transmission electron microscopy to penetrate other cells. Because sporozoites were still surrounded by an envelope containing host cell membranes, the requirement for contact between the tip of the sporozoite and the receptor on the host cell plasmalemma may have been fulfilled, enabling the sporozoite to

penetrate another cell. However, there would be no receptor contact on the plasmalemma of the host cell being penetrated by a host cell-enveloped sporozoite. Therefore, it appears that in some instances host cells may be receptive to purely mechanical penetration by zoites without the need of host cell–parasite receptor contact.

Penetration of an apicomplexan into a cultured cell does not necessarily result in multiplication of the parasite. After entry into host cells, the parasite and host cell must set up an environment conducive to parasite growth and development. When zoites of *T. gondii* penetrate into host cells, a tight junction is formed between the host cell and parasite at the site of penetration, inducing the release of rhoptry contents. Dense granules are also released during and shortly after penetration. Once inside the cell, a space develops between the host cell and the parasite; this is called the parasitophorous vacuole. Multiplication of the parasite appears to be dependent upon proteins from rhoptries and dense granules becoming incorporated into the parasitophorous vacuole membrane and the development of a tubulovesicular membrane network within the parasitophorous vacuole.

Immunity

The development of immunity is dependent upon the outcome of a complex and dynamic interplay between the host and the apicomplexan and is mediated through the host's immune system. The immune system changes adaptively in response to the activity and behaviour of the apicomplexan.

Both antibody-dependent and cell-mediated (CMI) mechanisms contribute to immunity in malaria as well as in other apicomplexans. Antigenic variation among malarial parasites results in considerable variation in their sensitivity to antibody-mediated inhibition. Both Ãã and T cells are important in malaria immunity. Although T cells may increase 40-fold or more in acutely infected humans and also inhibit growth of parasites *in vitro* and *in vivo*, their relative importance in protection or pathogenicity is still not clear. Both CD4 + and CD8 + Ãã T cells respond to malarial infection, but the role of CD8 + T cells in blood stage infections appears to be limited. CD4 + cells are of major importance and comprise at least two functionally different subsets (T_H1 and T_H2), with T_H1 cells producing primarily interleukin 2 (IL-2) and interferon gamma (IFN-) and giving rise to protection early in infections. T_H2 cells produce primarily IL-4 and are primarily responsible for clearance of parasites late in the infection. In areas of high endemicity of malaria, most humans have significantly elevated blood levels of IgE, but only approximately 5% of the IgE antibodies are directed against *P. falciparum*, indicating that there is skewing of the underlying T-helper cell ratio in favour of T_H2. IgE levels are highest in patients with cerebral malaria who also usually have elevated levels of tumour necrosis factor alpha (TNF-Ã). Localized overproduction of TNF-Ã is considered to be primarily responsible for fever and tissue lesions in severe falciparum malaria, but IgE also appears to contribute to the pathogenicity.

More than 60 years have been dedicated to searching for a malaria vaccine, yet there is still no effective means of immunizing against the disease. Four species infect humans: *Plasmodium falciparum*, *P. malariae*, *P. vivax* and *P. ovale*. Most of the effort in developing a vaccine has focused on *P. falciparum*, because it causes the most debilitating and lethal infections. Immunizing against malaria is complicated by the complexity of development in the human host, which involves several stages in red blood cells and in the liver: the sporozoite, the exoerythrocytic (EE) forms in the liver, and the asexual and sexual erythrocytic forms. Initially, efforts to develop a human malaria vaccine were directed against sporozoites or erythrocytic merozoites. However, it was discovered that immunizing against sporozoites failed to protect against erythrocytic merozoite infection. If a single sporozoite escaped immune detection, then a clinical infection could result.

It is now accepted that a multistage and multivalent vaccine will be needed to achieve an efficacious malaria vaccine. Compared to a single-stage antigen-based vaccine, a multistage and multivalent vaccine would be more efficacious by inducing multiple layers of immunity. Recent efforts have focused on developing genetically engineered vaccines consisting of genes encoding for as many as nine stage-specific antigens of *P. falciparum* corresponding to sporozoite, EE forms, and erythrocytic asexual and sexual stages. Preliminary studies have shown that such a multicomponent vaccine can induce immune responses that inhibit parasite development at multiple stages. Thus, an effective human malaria vaccine may be on the horizon.

Cryptosporidium parvum is a common source of diarrhoea in animals and usually causes a mild enteric disease in normal humans, and leaves the host solidly immune to reinfection. On the other hand, severe, chronic infections may develop in immunocompromised hosts. Infection induces specific IgM, IgG, IgA and IgE antibody responses, and transient local and secretory IgA antibody responses occur in the duodenum. Although the role of specific antibodies remains unclear, decreases in oocyst shedding in lambs and calves coincide with a rise in levels of coproantibodies, or antibodies in the intestinal contents. However, experimental studies in B-cell deficient mice do not suggest a protective role for either endogenous or secretory antibodies. Also, high antibody titres of IgG and IgM were found in many AIDS patients with chronic *C. parvum* infections. Nevertheless, colostrum from cows immunized against *C. parvum* decreases parasite burden and is moderately efficacious against *C. parvum* when given by mouth in AIDS patients with severe diarrhoea. Similar

examples of transmission-blocking immunity have been studied in poultry coccidiosis and malaria in mankind and have potential in the control of these diseases.

Most primary infections with *T. gondii* in immunocompetent hosts are clinically mild, but after the host recovers, the parasite may remain encysted in the central nervous system. Primary infection usually confers long-term protection against reinfection and it is rare for seropositive mothers to give birth to *T. gondii*-infected infants. Humoral antibodies react against several surface as well as cytoplasmic antigens. However, the CMI response to *T. gondii* represents the major component of host immunity which can be divided into an innate and a parasite-specific response. During acute infection, IL-2, produced by macrophages accelerates the proliferation of natural killer (NK) cells that release IFN-α to stimulate the release of nitrous oxide (NO). Although NO does not appear to be involved in acute infections, it is important in controlling parasite proliferation during chronic infections. After several days of infection, a parasite-specific T-cell response occurs, which provides long-term protection via the synergistic actions of CD4+ and CD8+ T cells. In addition to IFN-γ, a number of other cytokines are also important mediators of the CMI response, in particular IL-2, but IL-4, IL-6, IL-7 and IL-15 are also involved.

Major Taxa and Important Species

Class Perkinsea

Order Perkinsida. One family, one genus, *Perkinsus*.

Conoid forms an incomplete truncated cone; sexuality absent; with flagellated zoospores that have an anterior vacuole; monoxenous. *Perkinsus marinus*.

Class Gregarinea

Conoid present in sporozoite for a period of time, converted to mucron upon entry into host cell; merogony absent except in one group; gamonts large, extracellular, usually found in syzygy; gametes usually similar (isogamous); parasites of the digestive tract or body cavity of invertebrates and some lower chordates; monoxenous. Three orders: Archigregarinida, Eugregarinida, Neogregarinida. *Gregarina*, *Monocystis*, *Selenidium*, *Mattesia*.

Class Coccidea

All elements of the apical complex present; sporogony, merogony and gamogony present in all except two obscure orders.

Order Adeleina

Macro- and microgamont usually associated in syzygy during development; one to four microgametes; sporozoites enclosed in an envelope, monoxenous or heteroxenous. *Adelea*, *Haemogregarina*, *Klossiella*, *Hepatozoon*.

Order Eimeriina

Macro- and microgamonts develop separately; large number of microgametes formed; zygote nonmotile; no syzygy; sporozoites typically enclosed in a sporocyst which, in turn, is enclosed in an oocyst; large number of microgametes; monoxenous or heteroxenous. *Eimeria*, *Isospora*, *Tyzzeria*, *Sarcocystis*, *Toxoplasma*, *Frenkelia*, *Cyclospora*.

Order Cryptosporiina

Macro- and microgamonts develop separately; 16 or fewer merozoites and microgametes; sporozoites enclosed in a membrane that is either single or double; rhoptry-like bodies in zoite, small conoid in some stages; no micronemes or polar ring; sporogony within the vertebrate host; retrofection an important part of the life cycle; endogenous forms usually about 5 μm and lying under the plasma membrane of enterocytes, but extracytoplasmically; parasites of birds, reptiles and mammals; monoxenous. *Cryptosporidium*.

Class Haemosporea

Zoites have rhoptries, micronemes and polar ring, but lack a conoid; macro- and microgamonts develop separately, microgamont produces eight-flagellated microgametes; zygote motile (ookinete); sporozoite naked; heteroxenous with merogony in vertebrates and sporogony in invertebrates; transmission by blood-sucking insects. *Plasmodium*, *Leucocytozoon*, *Haemoproteus*.

Class Piroplasmea

Zoites have rhoptries, micronemes, and polar ring, and some species have a conoid; parasites of erythrocytes and leucocytes where the organisms are small rod-like, piriform, round or amoeboid forms; usually lacking microtubules; locomotion by body flexion, gliding, or in sexual stages by the large axopodium; sexual reproduction by syngamy; heteroxenous with merogony in vertebrates and gamogony and sporogony in invertebrates; sporozoites with single-membraned wall; ticks are the only known vectors, but vectors of dactylosomatids are unknown. *Babesia*, *Theileria*, *Dactylosoma*.

References

Marquardt WC, Demaree RS and Grieve RB (1999) *Parasitology and Vector Biology*, 2nd edn. San Diego: Academic Press.

Speer CA and Dubey JP (1999) Ultrastructure of schizonts and merozoites of *Sarcocystis falcatula* in the lungs of budgerigars (*Melopsittacus undulatus*). *Journal of Parasitology* **85**: 630–637.

Further Reading

Barta J (1989) Phylogenetic analysis of the class Sporozoa (phylum Apicomplexa Levine, 1970): evidence for the independent evolution of heteroxenous life cycles. *Journal of Parasitology* **75**: 195–206.

Clark DP and Sears CL (1996) The pathogenesis of cryptosporidiosis. *Parasitology Today* **12**: 221–225.

Corliss JO (1994) An interim utilitarian ('user-friendly') hierarchical classification and characterization of the protists. *Acta Protozoologica* **33**: 1–51.

Doyle PS, Crabb J and Petersen C (1993) Anti-*Cryptosporidium parvum* antibodies inhibit infectivity in vitro and in vivo. *Infection and Immunity* **61**: 4079–4084.

Fourmaux MN (1996) *Toxoplasma gondii* microneme proteins: gene cloning and possible function. *Current Topics in Microbiology and Immunology* **219**: 55–58.

Jensen JB (ed) (1983) *In Vitro Cultivation of Protozoan Parasites*. Boca Raton, FL: CRC Press.

Levine ND (1985) Apicomplexa. In: Lee JJ, Hutner SH and Bovee EC (eds) *An Illustrated Guide to the Protozoa*. Lawrence, KS: Society of Protozoologists.

Levine ND (1988) *The Protozoan Phylum Apicomplexa*. Boca Raton, FL: CRC Press.

Margulis L, Corliss JO, Melkonian M and Chapman DJ (1990) *Handbook of the Protoctista*. Boston: Jones & Bartlett Publishers.

Perlmann P, Perlmann H, Berzins K and Troye-Blomberg M (1998) Selected problems of malaria blood stage immunity. *Tokai Journal of Experimental Clinical Medicine* **23**: 55–62.